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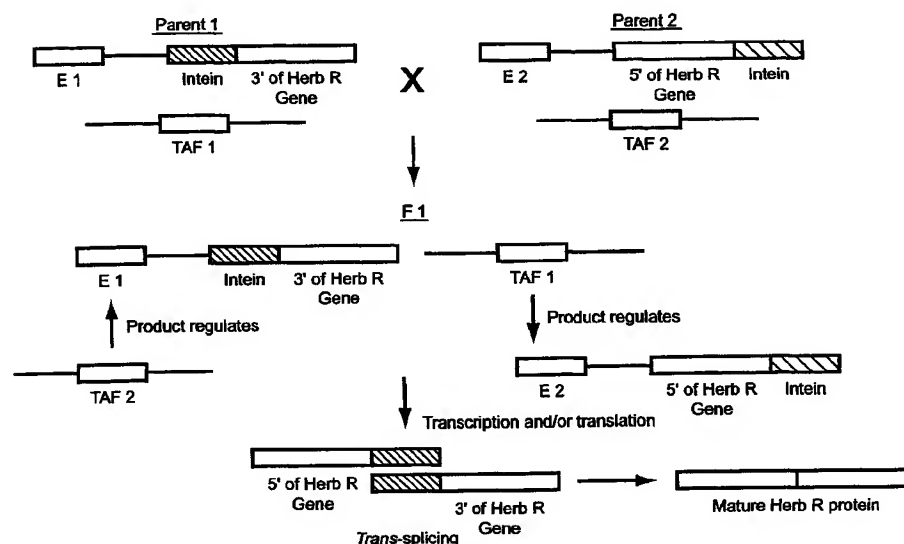
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IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,  
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,  
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[Continued on next page]

(54) Title: ENCRYPTION OF TRAITS USING SPLIT GENE SEQUENCES



(57) Abstract: Methods of unencrypting trait encrypted gene sequences to provide unencrypted RNAs or polypeptides. The invention also relates to methods of encrypting traits including splitting genes between two parental organisms or between a host organism and a vector. The gene sequences are unencrypted when the two parental organisms are mated or when the vector infects the host organism by *trans*-splicing either the split RNAs or split polypeptides upon expression of the split gene sequences. The invention also includes methods of providing multiple levels of trait encryption and reliable methods of producing hybrid organisms. Additional methods include those related to unencrypting engineered genetic elements to provide polypeptide functions and those directed at recombining non-overlapping gene sequences. The invention also includes integrated systems and various compositions related to the disclosed methods.



(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent  
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,  
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GW, ML, MR, NE, SN, TD, TG).

**(88) Date of publication of the international search report:**  
19 July 2001

**Published:**

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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05448

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/00 C12N9/14 C12N15/10 C12N15/62 C12N15/82  
A01H1/00 A01H5/00 G06F17/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 07351 A (HARVARD COLLEGE) 16 March 1995 (1995-03-16)  page 2, line 35 -page 3, line 7 page 23, line 5 -page 29, line 10 page 39, line 35 -page 41, line 34 page 6, last paragraph page 44 page 48 -page 49	1,2, 4-10,12, 54, 56-65, 106,107
A	---	15-20, 23, 31-40,53
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

20 November 2000

Date of mailing of the international search report

06. 2. 01

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/05448

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WU H ET AL: "Protein trans-splicing by a split intein encoded in a split DnaE gene of <i>Synechocystis</i> sp. PCC6803" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 95, August 1998 (1998-08), pages 9226-9231, XP002137477 WASHINGTON., US ISSN: 0027-8424 page 9228, last paragraph -page 9229	1,3,5-7, 11,54, 57,58, 63-65
A	---	16,17, 32,33
X	SOUTHWORTH M W ET AL: "Control of protein splicing by intein fragment reassembly" EMBO JOURNAL, OXFORD, GB, vol. 17, no. 4, 1998, pages 918-926, XP002121550 ISSN: 0261-4189 page 919 page 923, column 2 -page 924	1,3,5,6, 10,12
A	---	13,46
X	SHINGLEDECKER K ET AL: "Molecular dissection of the <i>Mycobacterium tuberculosis</i> RecA intein: design of a minimal intein and of a trans-splicing system involving two intein fragments" GENE, BARKING, GB, vol. 207, no. 2, 30 January 1998 (1998-01-30), pages 187-195, XP004108918 ISSN: 0378-1119 page 192, column 2 -page 193 page 195	1,3
A	---	11
A	WO 92 13089 A (GEN HOSPITAL CORP) 6 August 1992 (1992-08-06)  page 11, line 24 -page 12, line 2 page 28, line 32 -page 29, line 7 page 30, line 5 -page 31, line 20 figure 7	21,22, 24-30, 41-52, 108-110
A	---	46
	EVANS T C ET AL.: "Semisynthesis of cytotoxic proteins using a modified splicing element" PROTEIN SCIENCE, vol. 7, 1998, pages 2256-2264, XP002925638 US abstract  ---	
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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 00/05448

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	<p>WO 00 17342 A (UNIV BOSTON) 30 March 2000 (2000-03-30)</p> <p>abstract</p> <p>-----</p>	<p>1,2,4-6, 11,12, 106</p>

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US 00/05448

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: **118-119**  
because they relate to subject matter not required to be searched by this Authority, namely:  

The subject-matter of claims 118-119 was considered to represent a mere presentation of information in the sense of Rule 39.1(v) PCT, and a computer program in the sense of Rule 39.1(vi) PCT, and therefore no search has been carried out for those claims.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  

**1-66, 106-110 completely and 118-121 partially**

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## 1. Claims: 1-66, 106-110 completely and 118-121 partially

A method of unencrypting trait encrypted gene sequences to provide at least one unencrypted RNA or polypeptide comprising transcribing a plurality of split gene sequences to provide a plurality of RNA segments, trans-splicing at least two RNA segments together to provide an unencrypted RNA or translating the plurality of RNA segments to provide polypeptide segments and trans-splicing at least two polypeptide segments together to provide an at least one first unencrypted polypeptide; parental organisms, host organism, vectors, split gene sequence libraries and an integrated system comprising a computer for use in said method.

## 2. Claims: 66-105 completely and 118-121 partially

A method for unencrypting engineered genetic elements to provide at least one unencrypted polypeptide function comprising post translational modification of said encrypted polypeptide; genetic elements, parental organisms, host organism, vectors and an integrated system comprising a computer for use in said method and polypeptides and organisms made by said method.

3. Claims: 111-117 completely,  
118-121 partially and 122-123 completely

A method of recombining non-overlapping gene sequences comprising combining a plurality of gap nucleic acid sequences, which overlap two or more of the non-overlapping sequences, with a plurality of non-overlapping sequences; a composition comprising libraries of gap nucleic acids and an integrated system comprising a computer for use in said method.

# INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No

PCT/US 00/05448

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